# Quartz Crystal Microbalance Analysis of Growth Kinetics for Aggregation Intermediates of the Amyloid-β Protein

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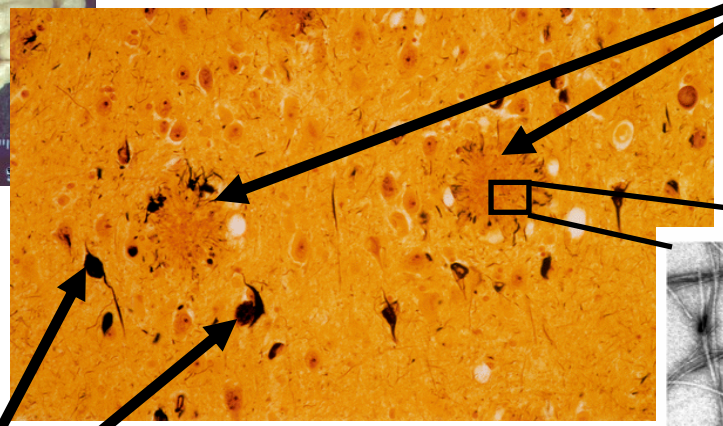
# Alzheimer's Pathology

## Alzheimer's Brain

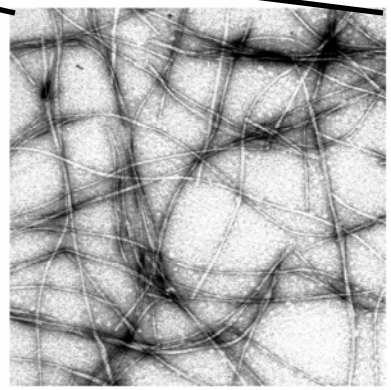
Courtesy of D. Dickson,
Mayo Clinic, Jacksonville.

## Pathology



Amyloid Plaques

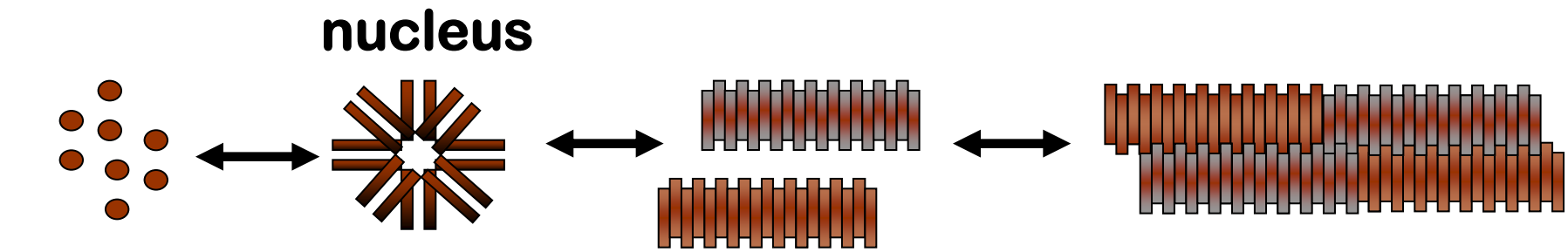
Aβ Fibrils

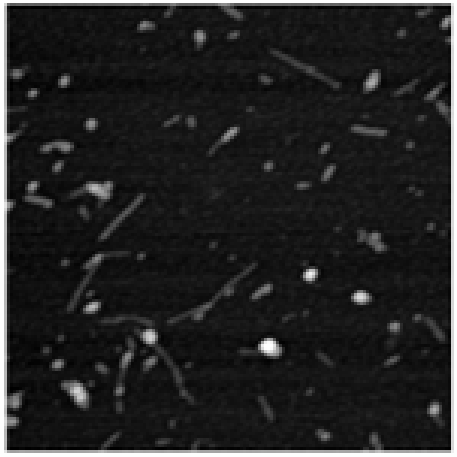


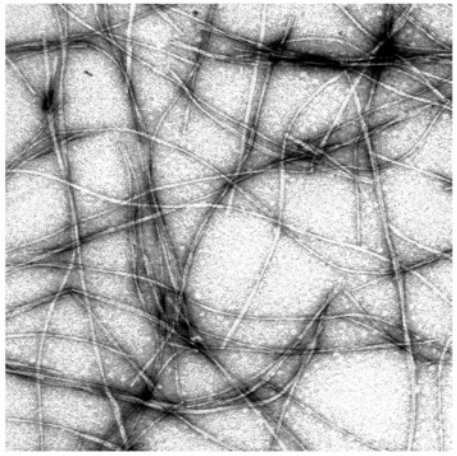
Neurofibrillary Tangles

Nichols *et al.* (2002)
*Biochemistry*, 41: 6115-27.

# How Does Aβ Assemble Into Fibrils?



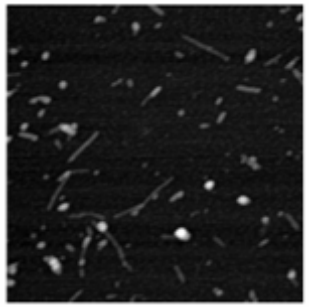





Nichols *et al.* (2002) *Biochemistry*, **41**: 6115-27.

# Pathogenic Role for Aggregation Intermediates

- **Capable of inducing physiological response**
  - Impaired hippocampal long-term potentiation
  - Synaptic loss
  - Neurotoxicity
  - Altered memory function



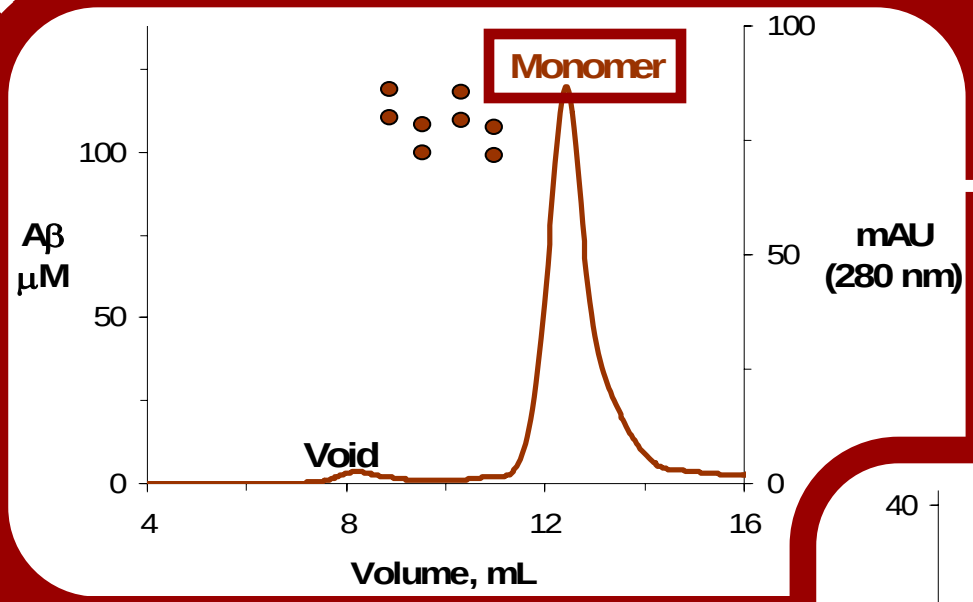
- **Active growth has been observed to induce toxicity**
  - Human cortical neurons
  - Cerebrovascular smooth muscle cells

# Key Research Questions…

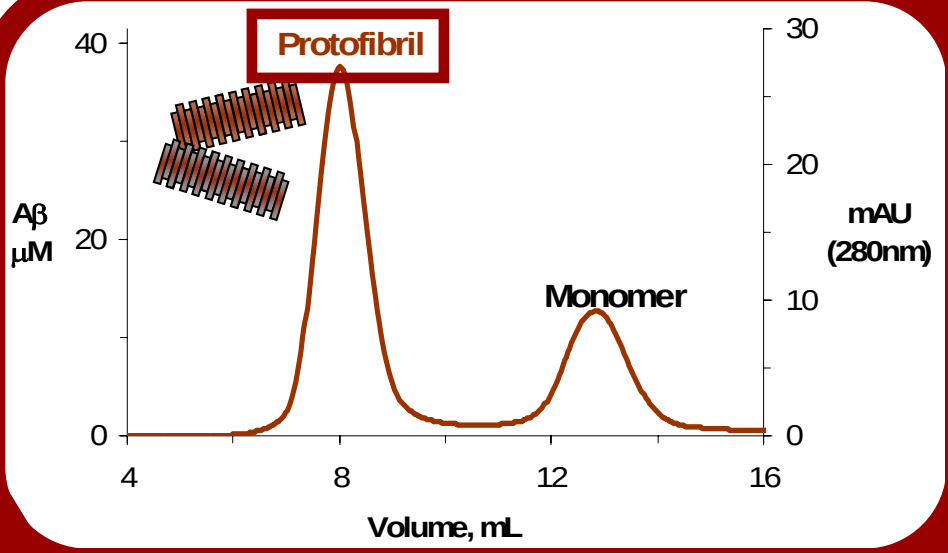
**What kinetics govern Aβ aggregation intermediate growth**

**Is growth of Aβ aggregation intermediates influenced by varying solution conditions**

# Isolation of Aβ Species




2-10 mM NaCl → Mixture



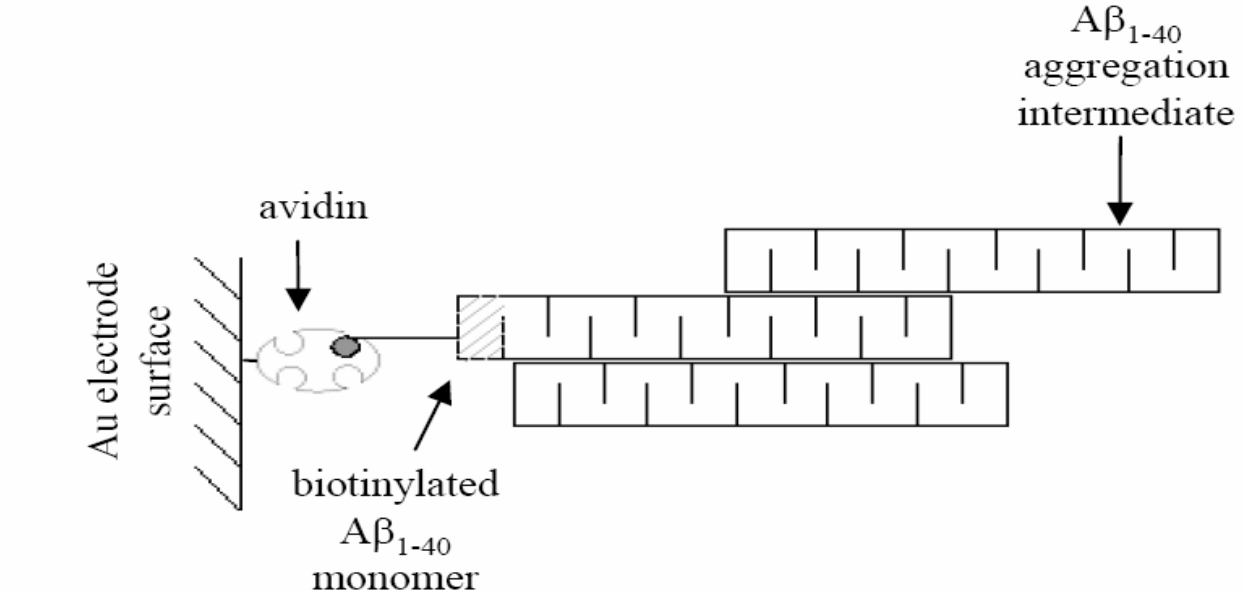
# Quartz Crystal Microbalance

- **Decrease in resonance frequency is related to an increase in bound mass**

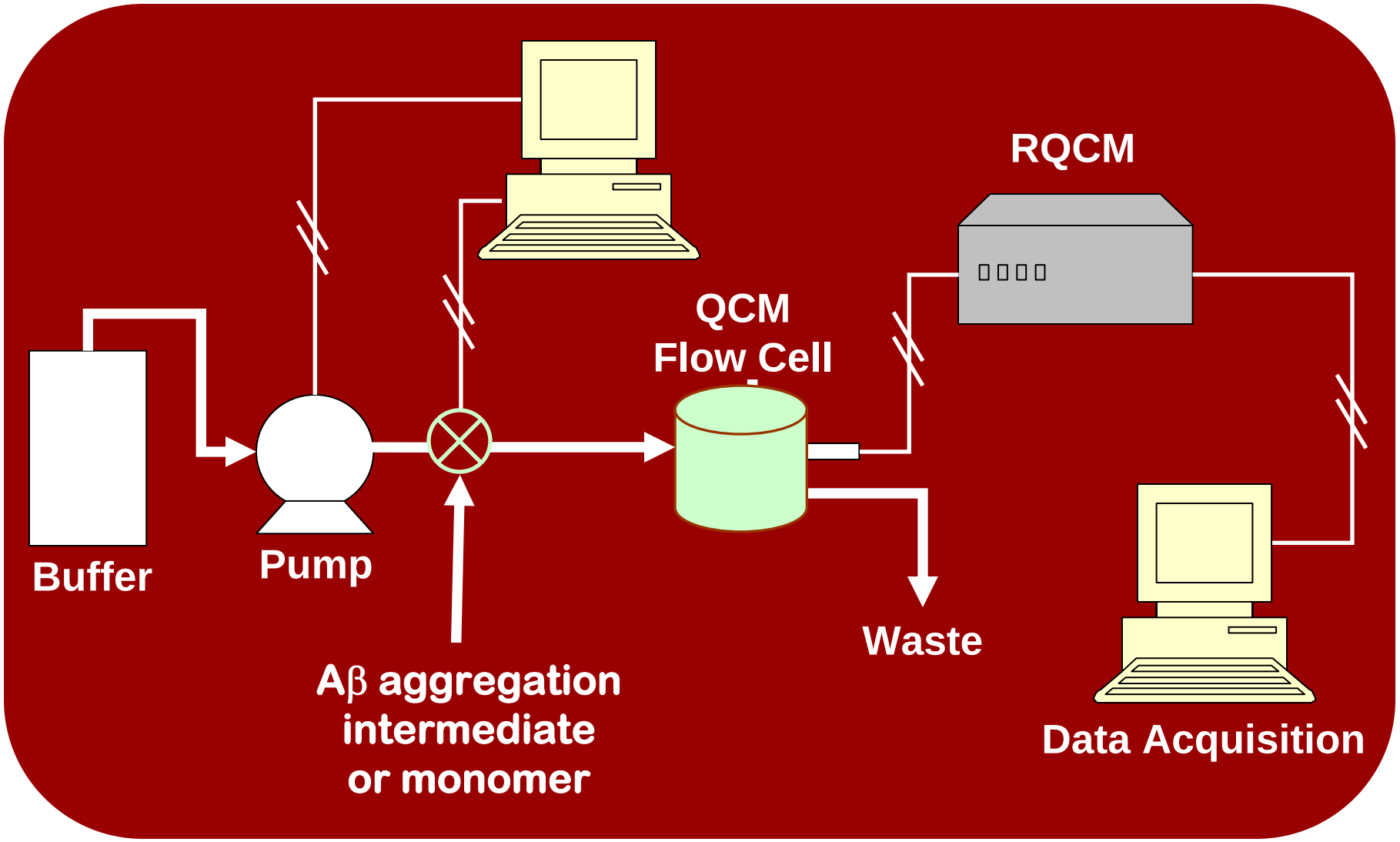
$$\Delta f = \frac{-2f^2}{A(\mu\rho_q)^{0.5}}\Delta m = -C_f \Delta m$$

# Protofibril Immobilization via Avidin-Biotin

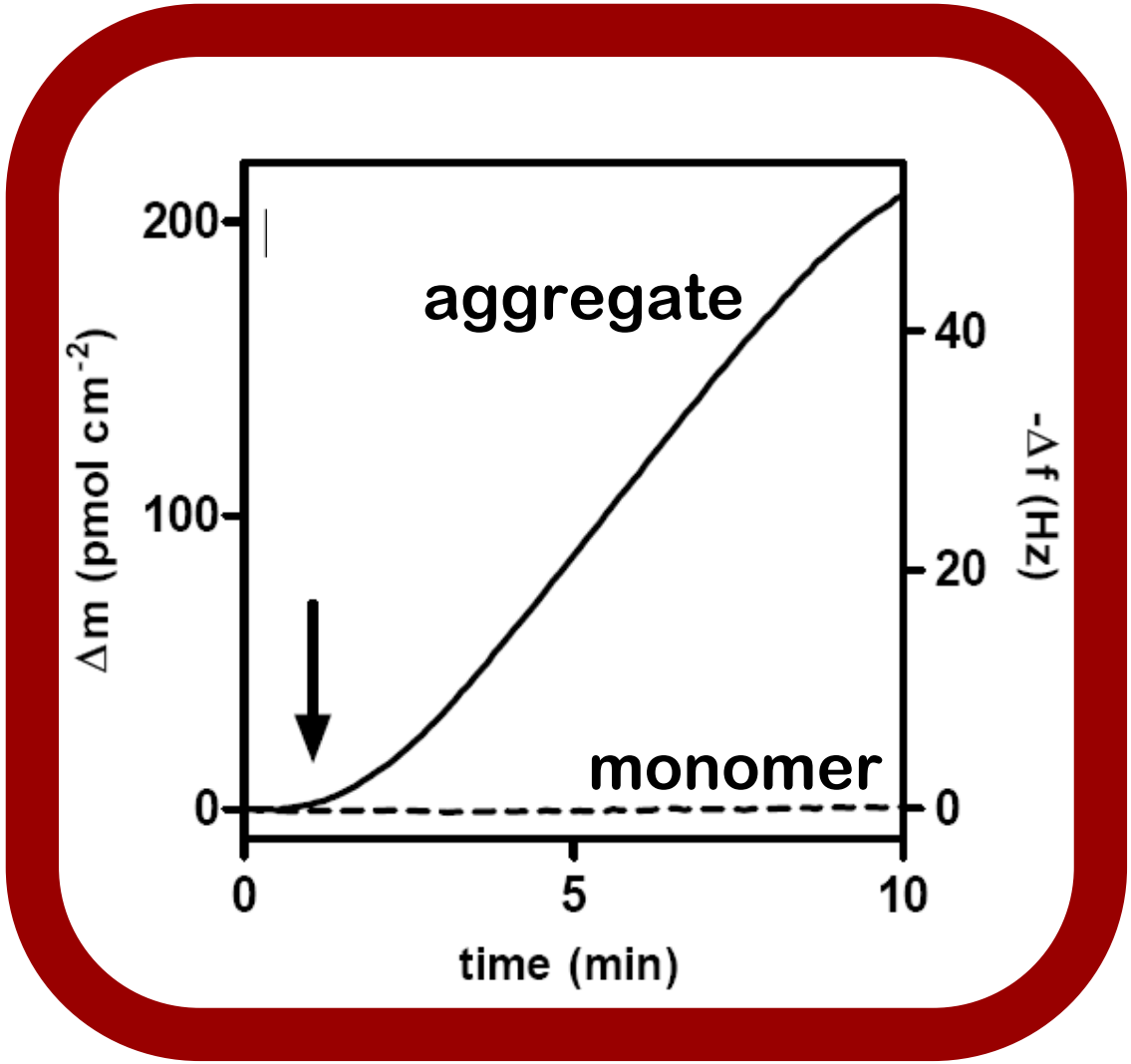
- Avidin was covalently coupled
- Biotinylated Aβ monomer bound avidin
- Unlabeled aggregation intermediates were immobilized as monomer was incorporated

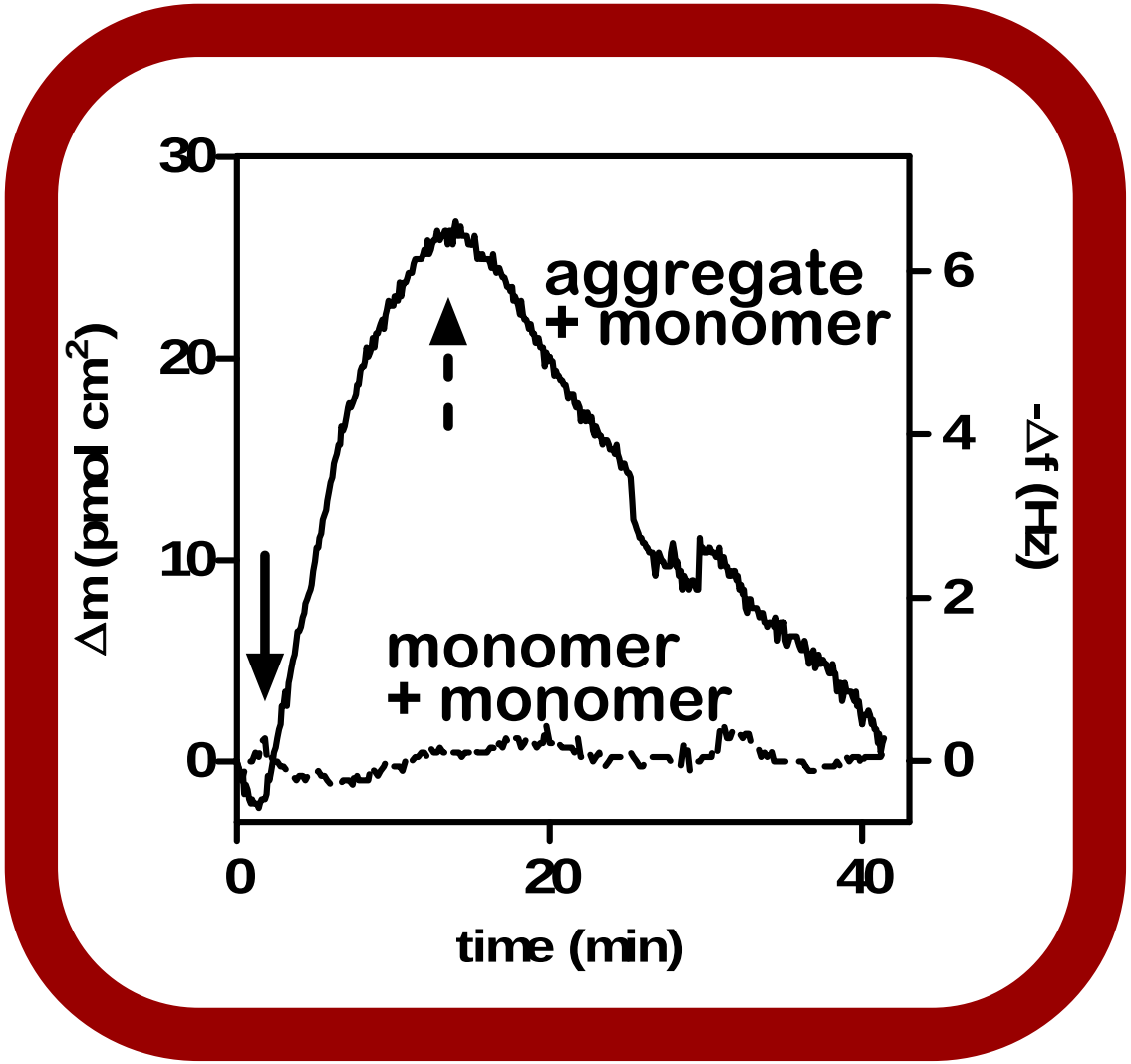


# Experimental Setup



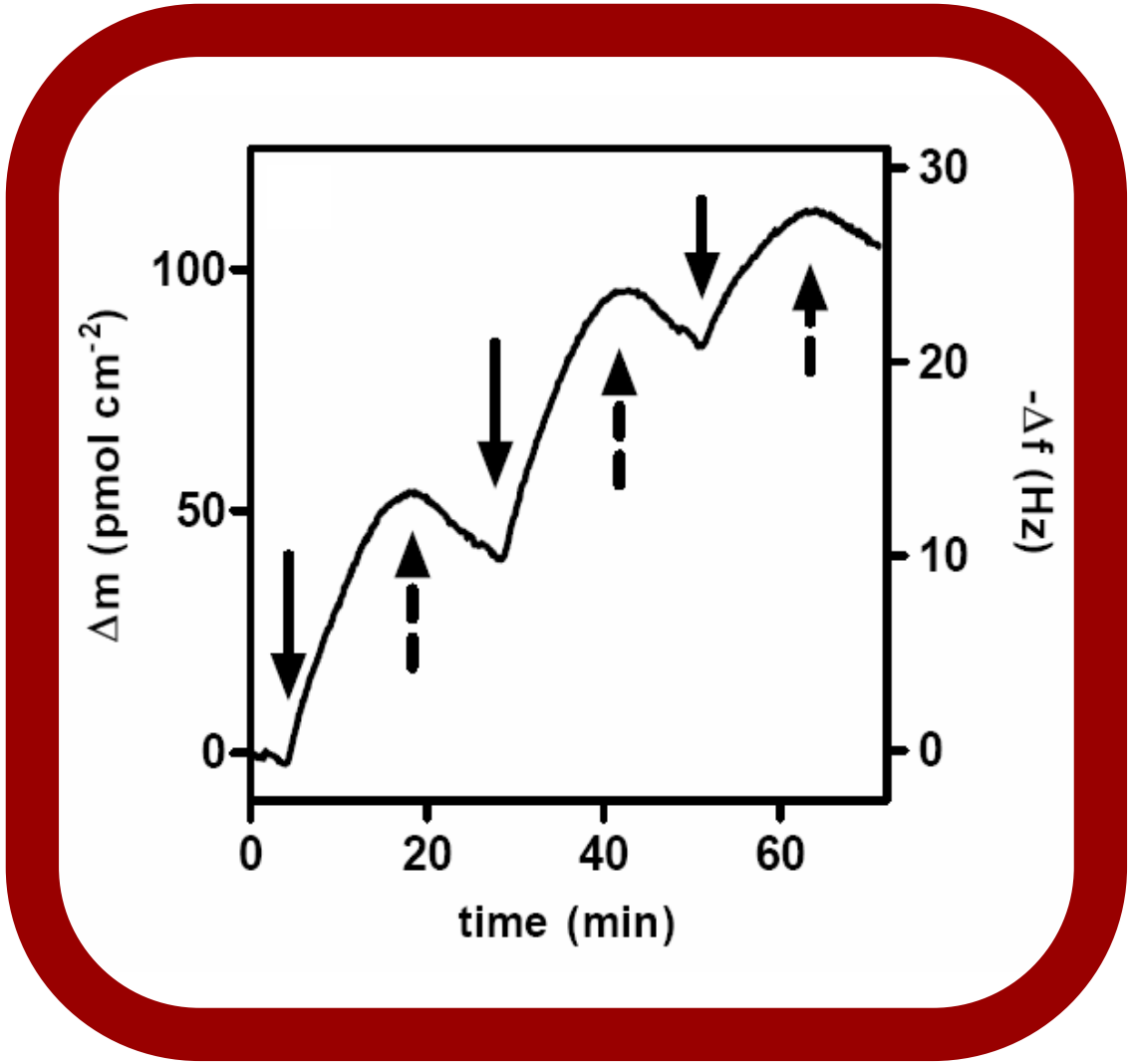
Aβ aggregation intermediates are selectively immobilized onto the crystal surface.

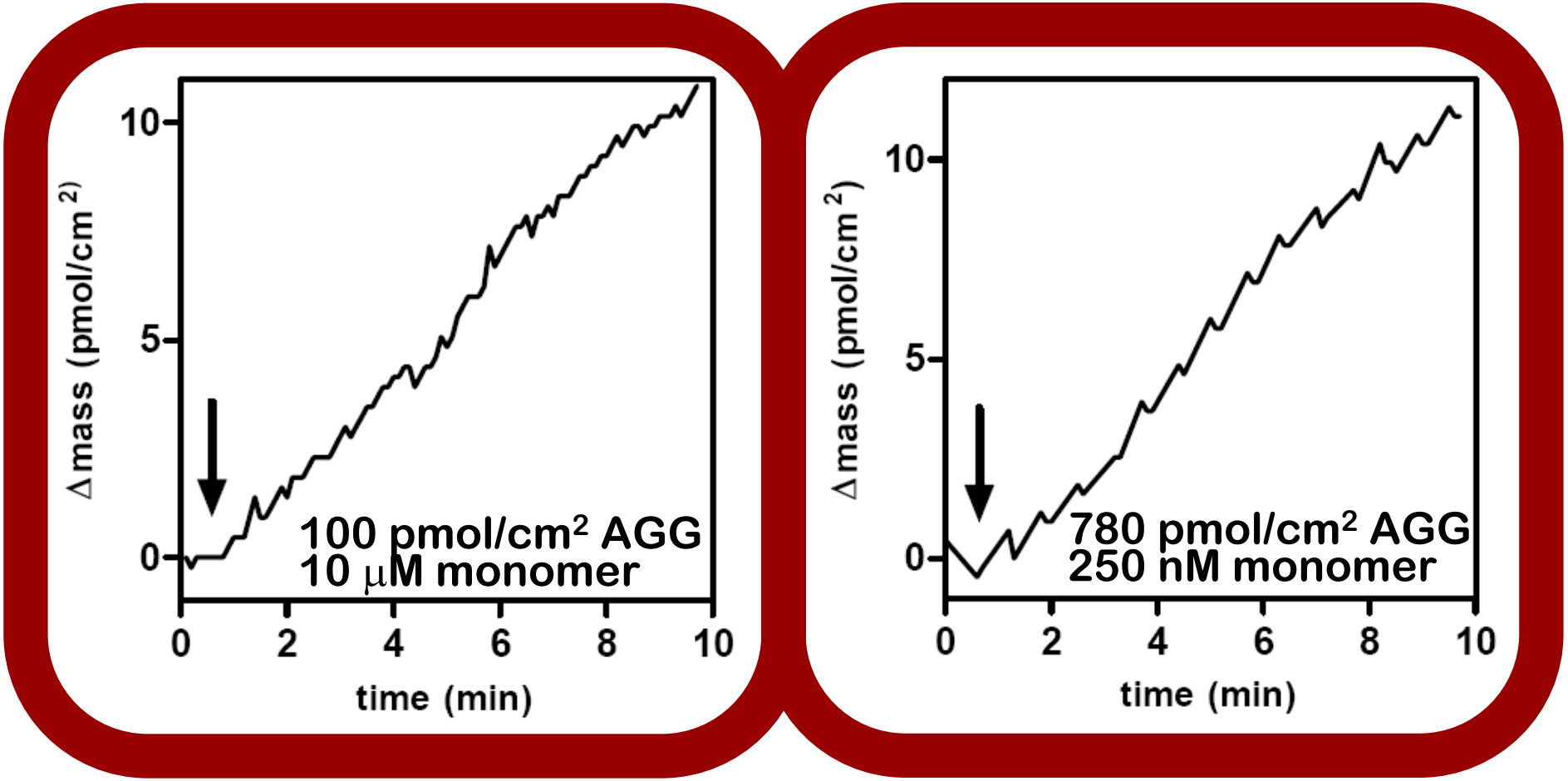




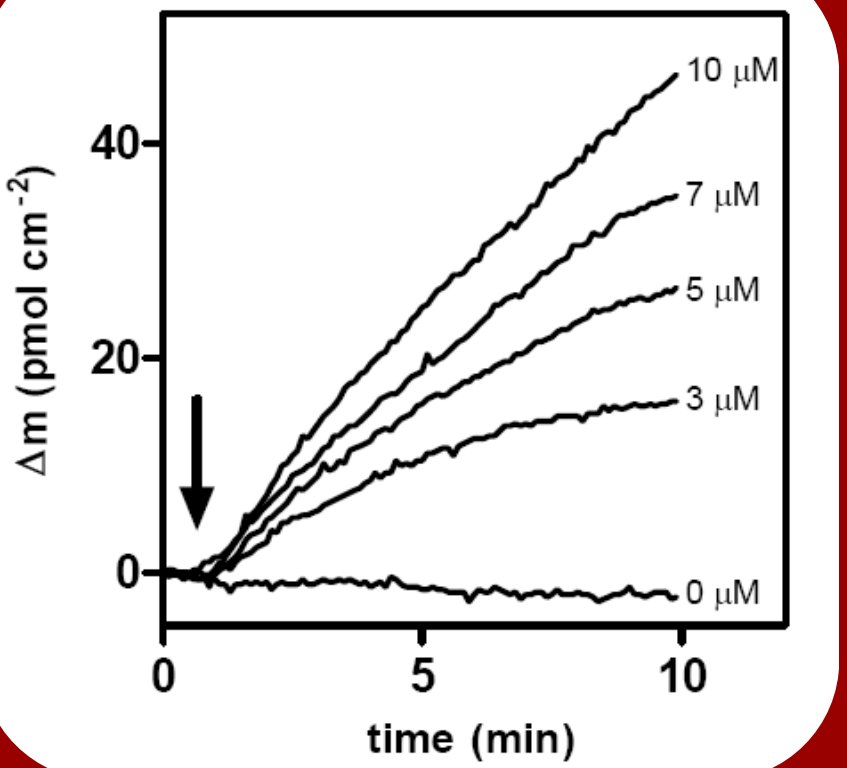

**Addition of Aβ monomer onto immobilized aggregation intermediates is successfully detected.**

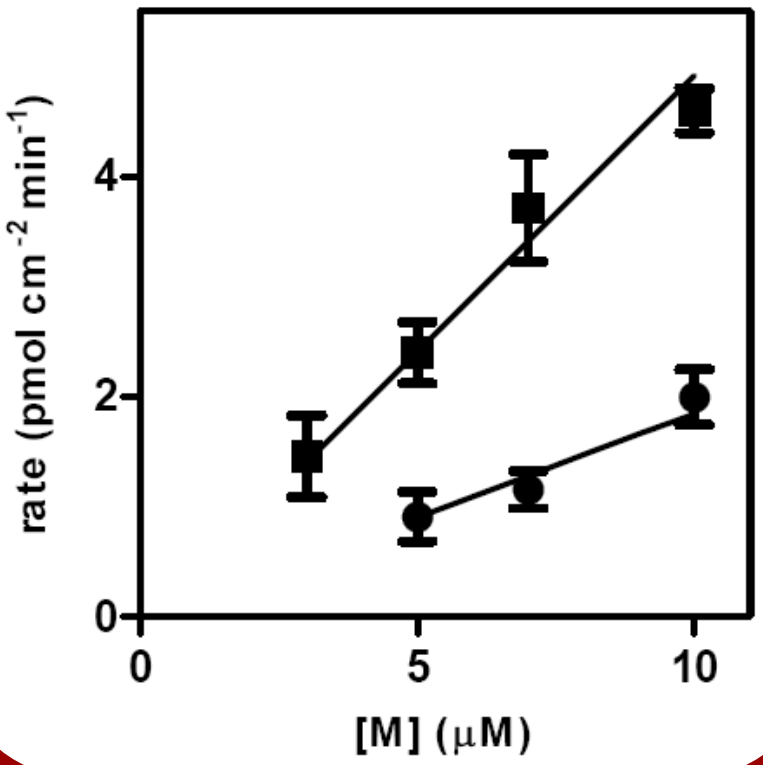
**Growth of immobilized aggregation intermediates is reversible and non-saturable.**



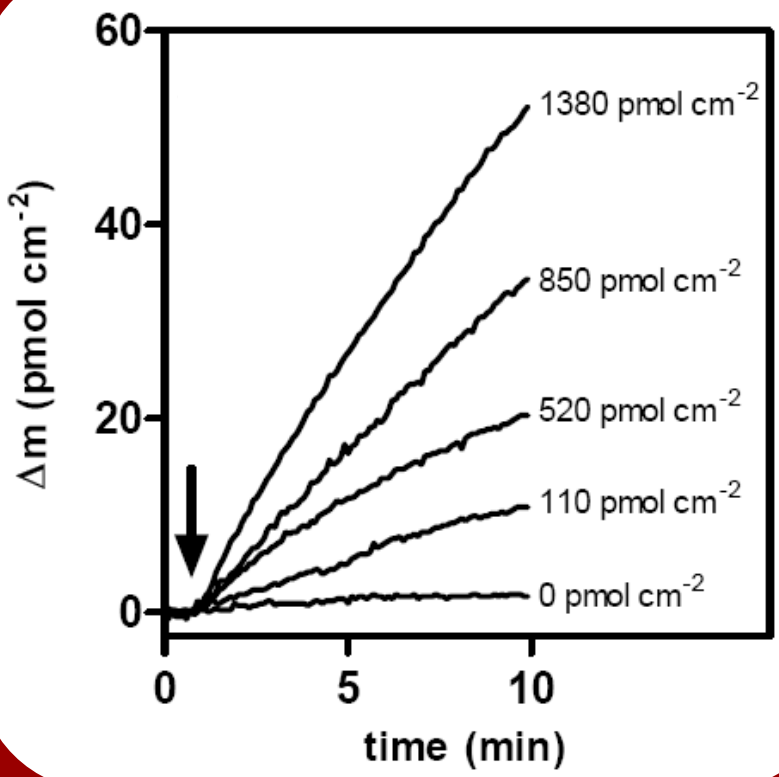


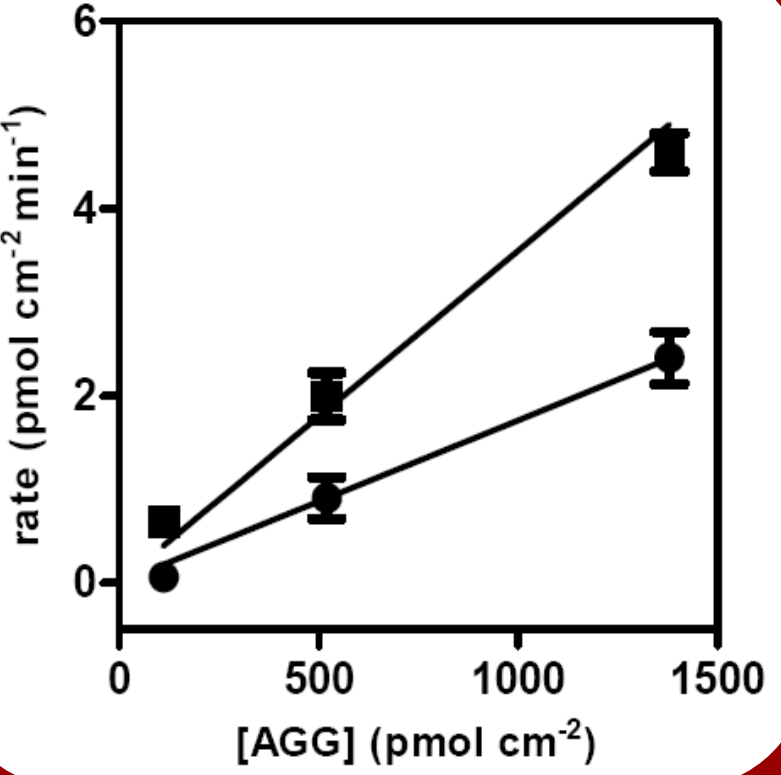

## Growth can be detected with high sensitivity.




## Growth is linearly dependent upon monomer…



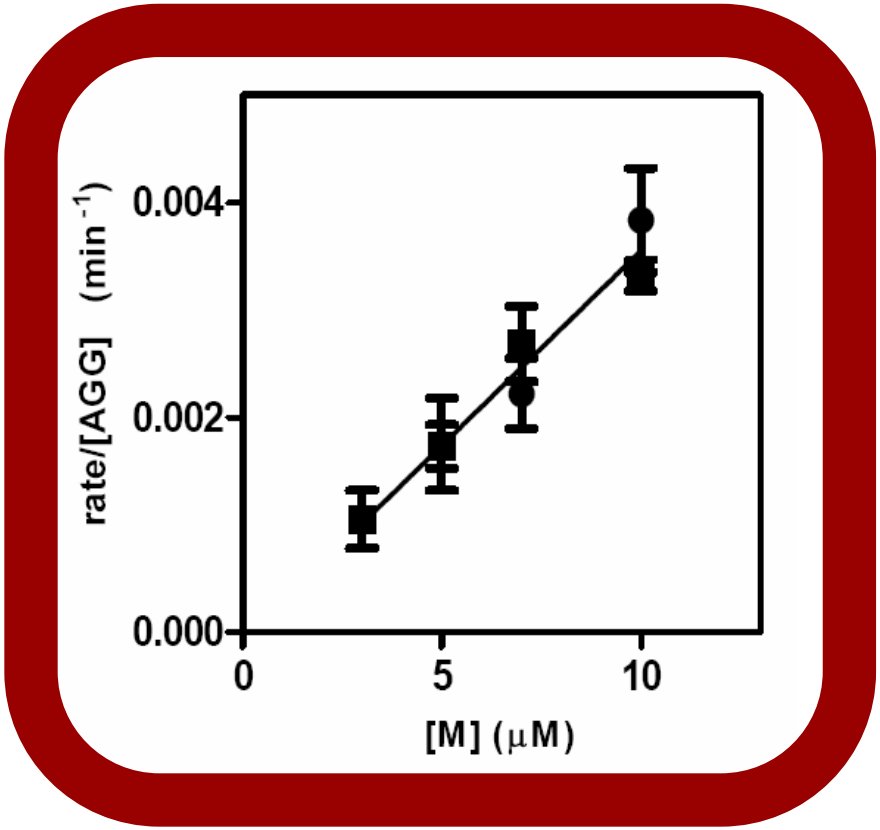




**…and immobilized aggregation intermediate density.**

## Growth is modeled via a first-order kinetic model.

$$[AGG]+[M] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [AGG]$$

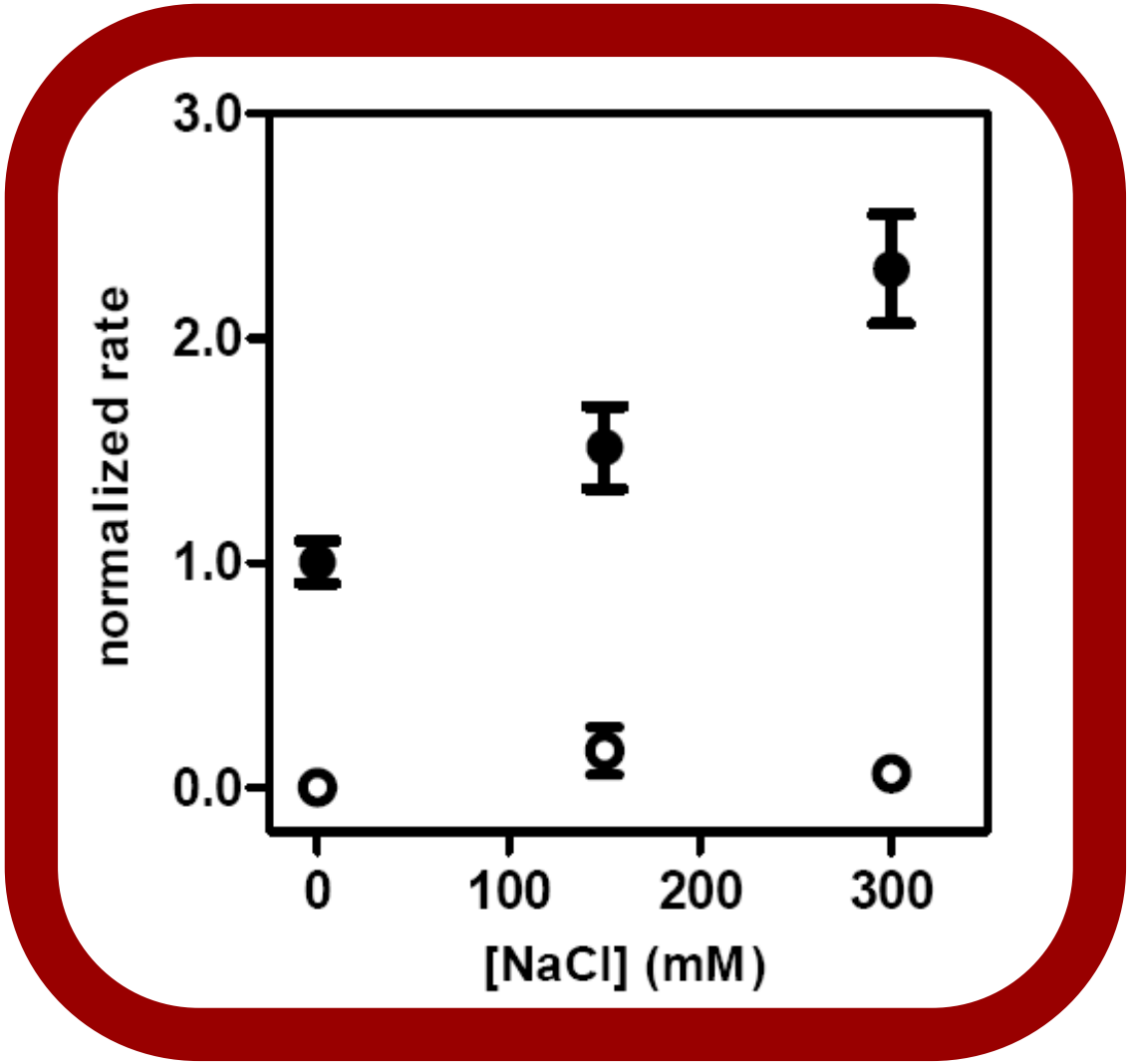
$$\frac{d[M]}{dt} = k_1[AGG][M] - k_{-1}[AGG]$$




$$k_1 = 3.6\times10^{-4}\ \mu\text{M}^{-1}\,\text{min}^{-1}$$

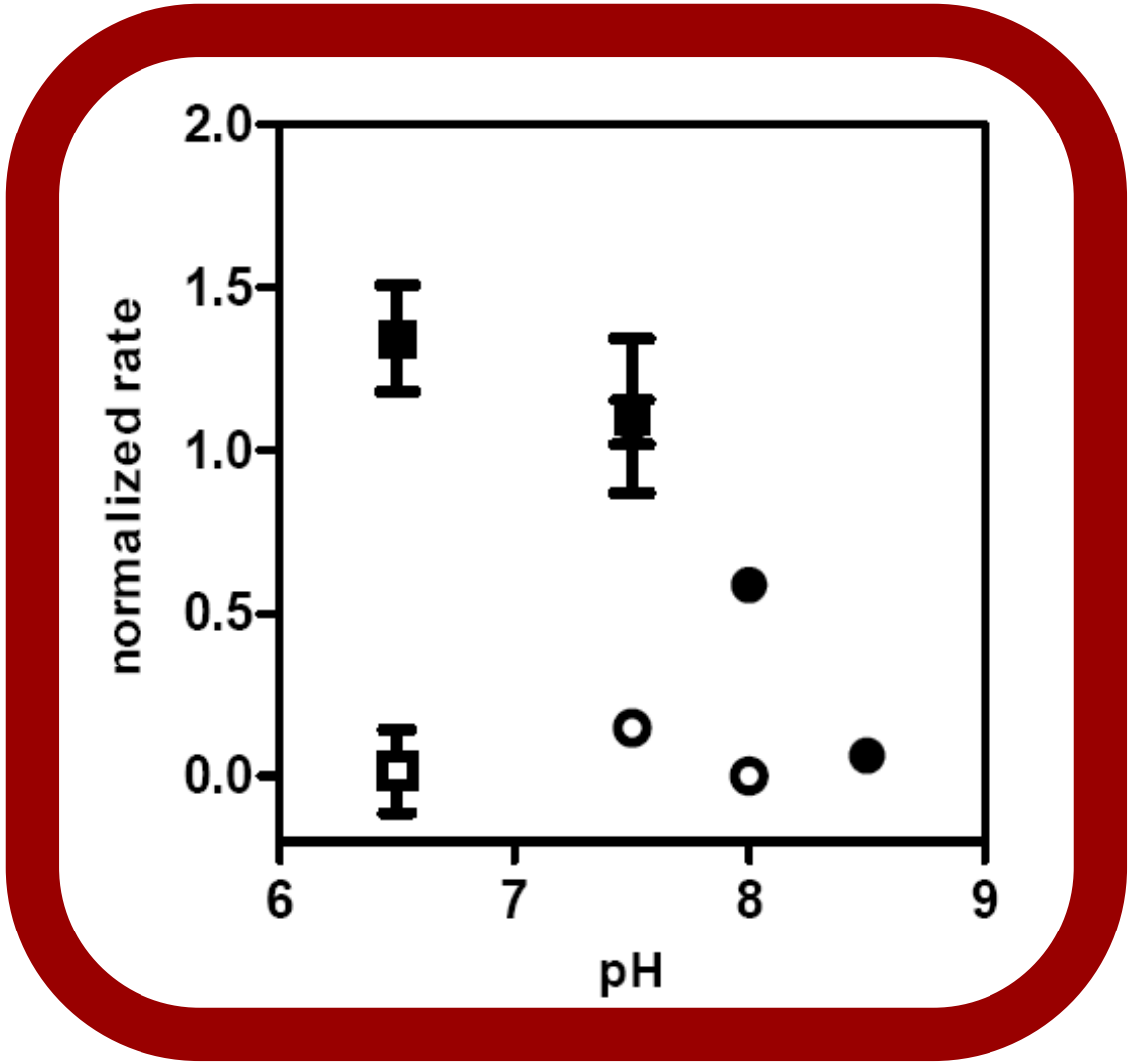
$$k_{-1} = 7.6\times10^{-5}\ \text{min}^{-1}$$

$$K_d = \frac{k_{-1}}{k_1} = 210\ \text{nM}$$




**Addition of Aβ monomer onto immobilized aggregation intermediates increases with increasing solution ionic strength.**

Addition of Aβ monomer onto immobilized aggregation intermediates increases as solution pH becomes more acidic.



# Summary

- Aβ aggregation intermediates can be selectively immobilized onto the QCM quartz crystal surface using avidin-biotin chemistry.
- QCM can be used to observe the growth of aggregation intermediates via monomer addition.
- Growth of Aβ aggregation intermediates can be described using a first-order kinetic model.
- Growth of Aβ aggregation intermediates is modulated by solution ionic strength and pH.

# Acknowledgements

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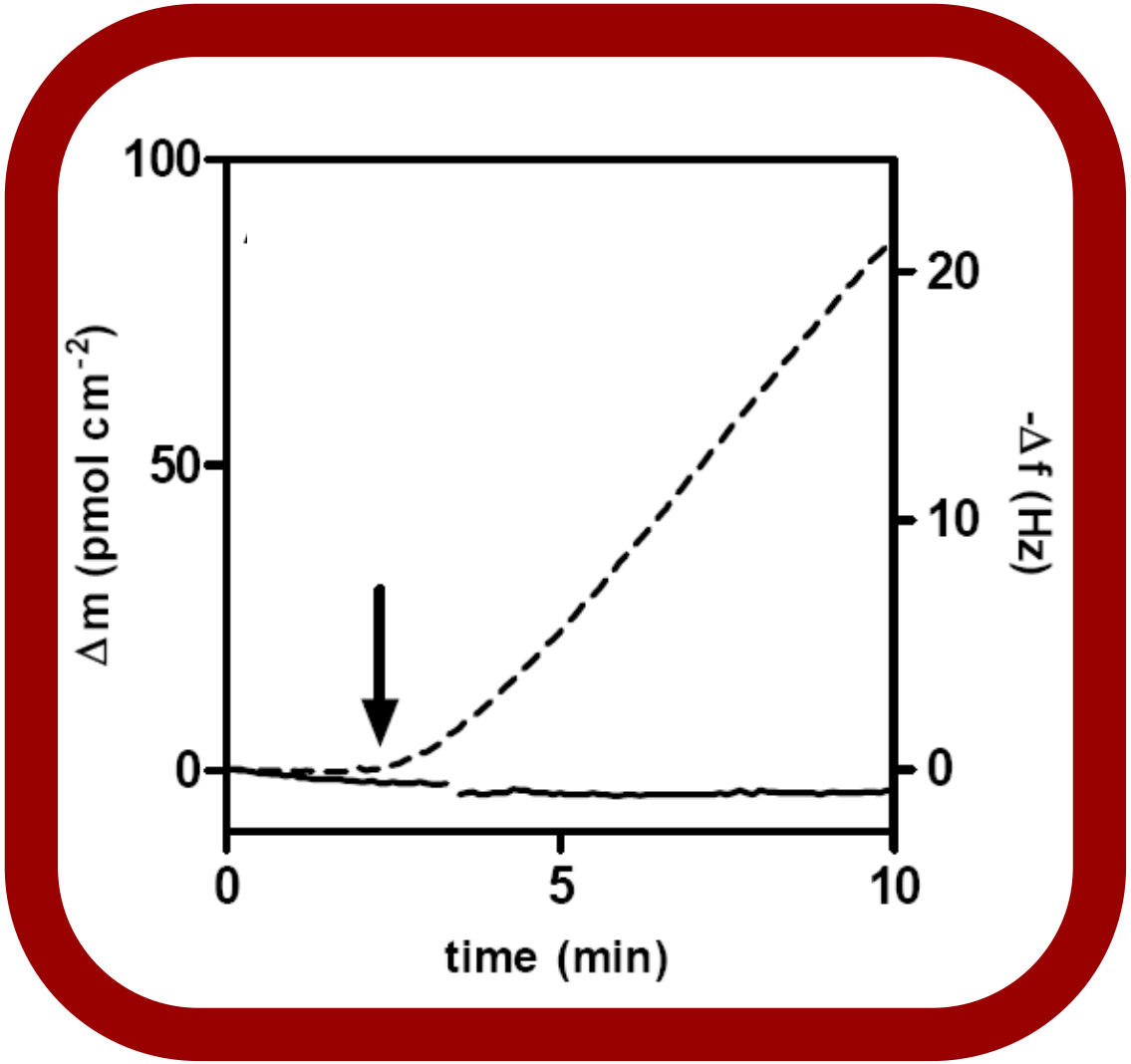
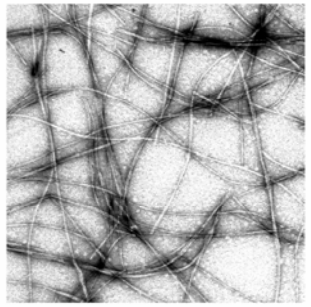



**A PEG-modified crystal surface significantly reduces non-specific binding of aggregation intermediates**

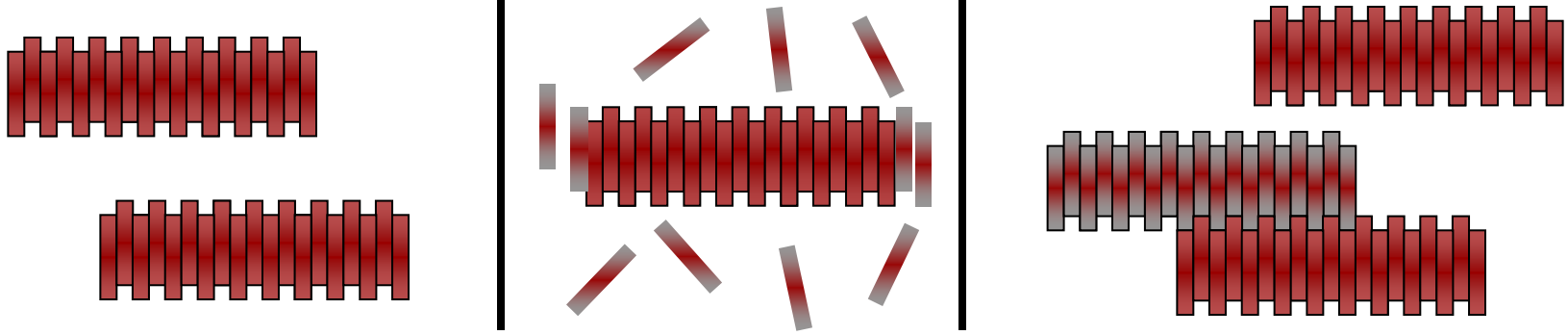
# Growth of Mature Aβ Fibrils

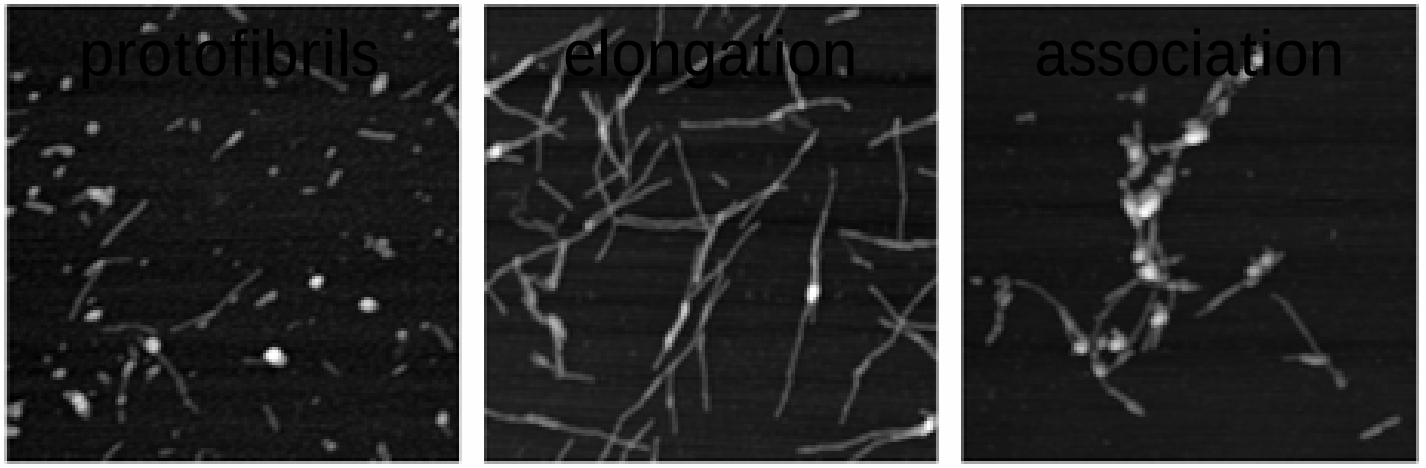


- **First-order kinetics**
  - Single growth phase
  - Non-saturable and reversible growth
- **"Dock-and-lock" model**
  - Two distinct growth phases
  - "locked" monomer dissociates slower than "docked" monomer

# Mechanisms of Growth for Aggregation Intermediates

**intermediates** | **elongation** | **association**



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